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Extra and intra-cranial blood flow regulation during the cold pressor test: influence of age

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D.F. contributed to the study design, data acquisition, data analysis, data interpretation and drafted the manuscript. A.R.B., K.W.W. and L.E.M. contributed to the data acquisition and critical review of the manuscript. P.N.A. contributed to the study design, data interpretation and critical review of the manuscript. J.P.F. contributed to the study design, data acquisition, data interpretation and drafted the manuscript. All authors approved the final version of the manuscript.

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Running head: Cold pressor test and cerebral blood flow

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ABSTRACT

We determined how the extra- and intra-cranial circulations respond to generalized sympathetic activation evoked by a cold pressor test (CPT) and whether this was affected by healthy aging. Ten young (23±2 yr; mean±SD) and nine older (66±3 yr) individuals performed a 3-min CPT by immersing the left foot into 0.8±0.3°C water. Common carotid artery (CCA) and internal carotid artery (ICA) diameter, velocity and flow were simultaneously measured (duplex ultrasound), along with middle cerebral artery and posterior cerebral artery mean blood velocity ($MCAv_{mean}$ and $PCAv_{mean}$), and cardiorespiratory variables. The increases in heart rate (~6 bpm) and mean arterial blood pressure (~14 mmHg) were similar in young and older groups during the CPT (P<0.01 vs. baseline). In the young group, the CPT elicited a ~5% increase in CCA diameter (P<0.01 vs. baseline) and tendency for an increase in CCA flow (~12%; P=0.08); in contrast, both diameter and flow remained unchanged in the older group. Although ICA diameter was not changed during the CPT in either group, ICA flow increased (~8%; P=0.02) during the first minute of the CPT in both groups. While the CPT elicited an increase in $MCAv_{mean}$ and $PCAv_{mean}$ in the young group (by ~20% and ~10%, respectively; P<0.01 vs. baseline), these intra-cranial velocities were unchanged in the older group. Collectively, during the CPT, these findings suggest a differential mechanism(s) of regulation between the ICA compared to the CCA in young individuals, and a blunting of the CCA and intra-cranial responses in older individuals.

New & Noteworthy

Sympathetic activation evoked by a cold pressor test elicits heterogeneous extra- and intra-cranial blood vessel responses in young individuals that may serve an important protective role. The extra- and intra-cranial responses to the cold pressor test are blunted in older individuals.

Keywords: Brain blood flow, elderly, sympathetic nerve activity

INTRODUCTION

The cold pressor test (CPT) has been widely employed for the assessment of human autonomic function (13, 17), peripheral vascular reactivity (7, 45, 53, 72) and cardiovascular risk stratification (6, 38, 61). However, the cerebrovascular responses to the CPT remain poorly understood, particularly in healthy aging and chronic disease. This issue is compounded by the controversy surrounding the sympathetic regulation of the extra- and intra-cranial blood vessels (1, 58). During the CPT, signals from activated cutaneous thermoreceptor and nociceptor afferents are rapidly integrated within the central nervous system (principally the hypothalamic and medullary regions) and lead to the activation of cortical sites (10). This activation elevates peripheral vascular resistance, HR and blood pressure (23) on account of the characteristic autonomic efferent response, consisting of a robust increase in sympathetic nerve activity (SNA) [e.g., increased plasma noradrenaline (19) and muscle sympathetic nerve activity (65)], and potentially a decrease in cardiac parasympathetic nerve activity [e.g., decreased HR variability (16)]. Cerebral blood flow may be affected by several mechanisms during the CPT, including neurovascular coupling, a hydraulic pressure effect even in the absence of a change in vascular resistance, local autoregulatory mechanisms, and by the sympathetic modulation of extra- and intra-cranial blood vessels.

In animal studies, innervation of the CCA, ICA and intra-cranial vasculature by postganglionic sympathetic nerve fibers has been identified (12, 37, 42); electrical stimulation of sympathetic nerves can evoke cerebral vasoconstriction (2, 66); and norepinephrine causes vasoconstriction in cerebral microvessels (36, 59). In humans, the spillover of noradrenaline from the brain into the internal jugular vein has been reported (43); clinically indicated upper thoracic sympathectomy increases ICA

85 diameter and flow (26); and stellate ganglion blockade reportedly increases cerebral
86 perfusion (62), although this is not been a universal finding (27). The effect of CPT-
87 evoked sympathoexcitation on cerebral perfusion has principally been evaluated in
88 terms of intra-cranial artery mean blood flow velocity and usually within the middle
89 cerebral artery ($MCAv_{mean}$). Intriguingly, both reductions (3, 41) and elevations (46,
90 47, 56, 73) in cerebral perfusion have been reported during the CPT, possibly due to
91 differences in the partial pressure of arterial carbon dioxide (P_aCO_2). With respects to
92 the regulation of extra-cranial blood flow during the CPT, an increase in common
93 carotid artery (CCA) diameter by ~8% is reported in young healthy individuals (28,
94 34, 53). In contrast, CCA diameter is reduced during the CPT in patients with
95 coronary artery disease, possibly due to the greater sensitivity of the α-adrenergic
96 receptors (53). Unfortunately, to date no assessment has been made of internal carotid
97 artery (ICA) diameter or volumetric flow during the CPT, but these are essential in
98 order to understand the implications for cerebral blood flow (as opposed to blood flow
99 to the head and scalp via the external carotid artery). It would seem unlikely that the
100 same responses were observed in the CCA and ICA during the CPT. In accordance
101 with Poiseuille's Law, small changes in diameter have a major effect on flow (e.g.,
102 flow α $(diameter/2)^4$). Accordingly, if the ICA were to dilate to a similar degree as the
103 CCA (e.g., ~8%) brain blood flow would increase markedly. Given that the brain
104 seems to be particularly effective at protecting itself from over-perfusion (68) and that
105 the ICA (and vertebral arteries) are known to be integral to the regulation of cerebral
106 blood flow through modifying vascular resistance (14, 22, 29, 39, 40), it seems
107 reasonable to expect that different responses occur in the CCA and ICA during the
108 CPT.

Increased age is associated with a multitude of structural, functional and regulatory alterations throughout the cardiovascular system (30, 31), including the brain (5, 54). Age-related increases in arterial stiffness (28, 33), impairments in endothelial vasodilator function and altered α- and β-adrenergic receptors signaling within the peripheral vasculature have been identified in humans (4, 11). However, the extent to which age modifies the cerebral blood flow responses to sympathetic stimulation remains unclear.

The purpose of this study was two-fold. First, to comprehensively describe the extra- (CCA, ICA) and intra-cranial (MCA) blood flow responses to the CPT. Second, to ascertain the influence of age on these cerebrovascular responses to the CPT. To achieve these goals, in both younger and older subjects, simultaneous measurements of CCA and ICA diameter, velocity and flow were made along with $MCAv_{mean}$ and posterior cerebral artery mean blood flow velocity ($PCAv_{mean}$) during the CPT under conditions of controlled isocapnia. We hypothesized that there would be less of an increase in ICA diameter compared to the CCA during the CPT in young individuals. In addition, we anticipated that the extra- and intra-cranial responses to the CPT would be blunted in older individuals.

MATERIALS AND METHODS

Ethical Approval

All experimental protocols and procedures were approved by the University of British Columbia Research Ethics Board (H15-01951) and conformed to the Declaration of Helsinki. Prior to participation a detailed verbal and written explanation of the study was provided and each participant completed written informed consent.

Participants

Nineteen study participants, ten young (2 women, 23±2 years, 176±7 cm, 73±9 kg, mean±SD) and nine older (2 women, 66±3 years, 176±8 cm, 78±13 kg) took part in the study. As determined by a written screening questionnaire and oral confirmation, no study participants had a history of cardiovascular, cerebrovascular or respiratory disease. None of our participants were active smokers, except one of the older participants had a history of smoking. Participants were not taking prescription or over-the-counter medications, except for two of the older male study participants who were using either Tamsulosin (0.4mg/day) due to enlarged prostate or Ciclesonide (400μg/day) due to mild asthma, and the two young women who were taking oral contraceptives and were tested on day 1 and 2 of their self-reported menstrual cycle. The two older women were both postmenopausal and not taking hormone replacements. Participants abstained from alcohol, caffeine and exercise for at least 12 hr prior to the experimental session.

Experimental measures

Cardiorespiratory measures

Heart rate (HR) was assessed using a 3-lead electrocardiogram (ECG; ADI BioAmp ML132), and beat-to-beat blood pressure using a finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands). Mean arterial pressure (MAP) was calculated from the Finometer reconstructed brachial waveform after values were back calibrated to the average of three automated brachial blood pressure measurements made over 3-min (Tango+; SunTech, Morrisville, NC). Stroke volume (SV) was estimated using the Modelflow

159 method (FMS, Amsterdam, The Netherlands), which simulates aortic flow waveforms
160 from an arterial pressure signal using a non-linear three-element model of the aortic
161 input impedance. Cardiac output (CO) was calculated as SV x HR, and total
162 peripheral resistance (TPR) as MAP / CO. Both the partial pressure of end-tidal CO_2
163 ($PetCO_2$) and O_2 ($PetO_2$) were sampled at the mouth and recorded by a calibrated gas
164 analyzer (model ML206, ADInstruments). A pneumotachograph (model HR 800L,
165 Hans Rudolph, Shawnee, KS) connected to a bacterial filter was used to assess minute
166 ventilation (VE). All cardiorespiratory variables were sampled continuously at 1000
167 Hz using an analogue-to-digital converter (Powerlab, 16/30; ADInstruments,
168 Colorado Springs, CO, USA) and data were interfaced with LabChart (Version 7), and
169 analyzed offline.
170
171 *Cerebrovascular measures*
172 Transcranial Doppler Ultrasound (2MHz, TCD, Spencer Technologies,
173 Seattle, WA) was used to simultaneously assess the right $MCAv_{mean}$ and left
174 $PCAv_{mean}$, in accordance with standard guidelines (67). A 2 MHz wavelength
175 provides the optimal resolution-to-penetration depth ratio for imaging the deep
176 cerebral vessels. The transmitted ultrasound beam contacts the red blood cells within
177 the target vessel and a portion of the signal is reflected back to the transducer.
178 The difference between the emitted and received frequency signals (i.e., Doppler
179 shift) is processed through a fast Fourier transformation to produce a velocity trace
180 and an envelope surrounding this is then exported in real time into LabChart (Version
181 7) for offline analyses. For anatomical reasons, in two older individuals the
182 orientation was switched such that the left $MCAv_{mean}$ and right $PCAv_{mean}$ were
183 insonated. Despite switching side in one individuals a clear image was impossible,

therefore $PCAv_{mean}$ is based on n = 8. The bilaterally placed probes were secured in place by being attached to a headpiece (model M600 bilateral head frame, Spencer Technologies). The MCA and PCA were insonated through the middle trans-temporal window, using previously described locations and standardization techniques (67). Blood velocity and vessel diameter of the left common carotid artery (CCA, right CCA n=3) and right internal carotid artery (ICA, left ICA n=3) were measured using a 10 MHz multi-frequency linear array vascular ultrasound (Terason T3200, Teratech, Burlington, MA). Due to anatomical reasons a clear image of the target artery was not possible in three study participants and therefore the side of insonation was switched. Only in two study participants were the ICA and MCA insonated contralaterally. B-mode imaging was used to measure arterial diameter, while pulse-wave mode was used to simultaneously measure peak blood velocity. Extracranial blood flow measurements were made in accordance with recent technical recommendations (60). All CCA and ICA recordings were screen captured and stored as video files for offline analysis (70). A minimum of 10 consecutive cardiac cycles were used to determine extracranial blood flow measurements. In 2 older study participants ICA images were on insufficient quality, thus ICA analysis in this cohort is based on n=7. Volumetric blood flow was calculated using the following formula:

$$\text{CCA or ICA flow} = \frac{\text{CCA or ICA Peak Envelope Velocity}}{2} \cdot [\pi\ (0.5\ \cdot \text{Diameter})^2]$$

Cerebrovascular conductance (CVC) was calculated for intracranial arteries and extracranial arteries using the following formula:

$$\text{MCA, PCA, CCA or ICA CVC} = \frac{\text{MCA}v_{mean}, \text{PCA}v_{mean}, \text{CCA flow or ICA flow}}{\text{MAP}}$$

Several indices of CCA and ICA stiffness were calculated in accordance with recently published methods (33, 34). β-stiffness index = ln(SBP-DBP)/[(DIAsys-DIAdia)/DIAdia], Elastic modulus = [(SBP-DBP)·DIAdia]/(DIAsys-DIAdia), arterial compliance = (DIAsys-DIAdia)/(SBP-DBP) and arterial distensibility = (DIAsys-DIAdia)/[(SBP-DBP)·DIAdia], where SBP; systolic blood pressure, DBP; diastolic blood pressure, DIAmax; maximum diameter and DIAmin; minimum diameter.

Study protocol

Study participants visited the laboratory on a single occasion. Prior to instrumentation all study participants were carefully familiarized with the study design and measurements. Thereafter the carotid, internal carotid and vertebral arteries were scanned in each participant in order to exclude individuals with any stenosis. After instrumentation and a resting period of at least 5 min, a 3-min baseline was recorded prior to the start of the CPT. The CPT consisted of a 3-min immersion of the left foot into ice cold water (0.8±0.3°C) followed by a 3-min recovery. The foot was chosen, rather than the hand, in order to keep the upper body still and facilitate the acquisition of high quality ultrasound images. Throughout the CPT, isocapnia was maintained using an end-tidal forcing system (Air-force, GE Foster, Kelowna, BC, Canada) described in detail elsewhere (49). Briefly, $PetCO_2$, $PetO_2$, inspiratory and expiratory tidal volume were sampled on a breath-by-breath basis and with the help of a feedback control, and using independent gas solenoid valves for O_2, CO_2 and N_2, desired end-tidal gases were maintained at baseline values. In order to assess whether there are any age-related alterations in thermal perception which may subsequently

contribute to any differences in CPT responses, each study participant was asked to provide a rating of the perceived pain, experienced at the onset and the end of the CPT using a Borg scale ranging from 0 (no pain) to 10 (worst pain).

Data and statistical analysis

Baseline (BL) values for the cardiovascular, respiratory and cerebrovascular variables measured were taken as an average over the last minute of the resting phase prior to the CPT. Thereafter, the last 20s of each minute was averaged during the CPT (CPT1, CPT2, CPT3) and throughout recovery (RE1, RE2, RE3). A repeated two-way ANOVA, was used to test for differences in the cardiovascular, respiratory and cerebrovascular responses with respects to experimental phase (BL, CPT1, CPT2, CPT3, RE1, RE2, RE3) and age (young, older). Data were expressed in absolute terms and as a percentage change from baseline, thus permitting us to compare the extra- and intra-cranial responses to the CPT and to ascertain the influence of age on these cerebrovascular responses. A repeated two-way ANOVA was used to determine whether perceived pain responses to the CPT were different with respects to experimental phase (CPT1, CPT2) and age (young, older). Finally, the existence of differences in arterial stiffness between experimental phases (BL, CPT) and age (young, older) was evaluated using a repeated two-way ANOVA. Tukey post hoc tests were used to examine significant main effects and interactions. Data are given as mean ± S.D unless otherwise indicated. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SAS Enterprise Guide (4.3, SAS Institute, Cary, NC).

RESULTS

Cardiovascular and respiratory variables during baseline, CPT and recovery in young and older participants are presented in Table 1. During the CPT, MAP increased from baseline in both groups ($P<0.01$), but absolute values were higher in the older group throughout ($P=0.03$). In both groups, HR was increased similarly at CPT1 ($P<0.01$ vs. baseline) and declined thereafter. The $PetCO_2$ was successfully kept at baseline values during the CPT by the end-tidal forcing system. Rating of perceived pain was not different between young and older groups at the onset (Young: 5.8±1.4, Older: 4.6±2.6) and the end of the CPT (Young 4.4±2.1, Older: 5.4±2.6).

During the CPT, $MCAv_{mean}$ and $PCAv_{mean}$ increased in the younger participants (by 19±19 and 11±12% at CPT2, respectively), whereas no changes from baseline were observed in the older participants (Figure 1). The CCA diameter increased in the young during CPT (by 5±3% at CPT1), whereas no change from baseline in CCA diameter was observed in the older participants ($P<0.01$, Figure 2). No changes from baseline in CCA velocity were observed in either age group, while CCA flow tended ($P=0.08$) to be increased in the young group. Both ICA diameter and ICA velocity were unchanged from baseline during the CPT, while ICA flow was increased from baseline at CPT1 ($P=0.03$). During the CPT, the percentage increase in CCA flow and $MCAv_{mean}$ were significantly greater than ICA flow in the young group (CCA vs. ICA $P=0.02$, CCA vs. MCA $P=0.70$, ICA vs. MCA $P<0.01$; Figure 3). However, in the older group the percentage increase in ICA flow and $MCAv_{mean}$ were significantly greater than CCA flow (CCA vs. ICA $P=0.02$, CCA vs. MCA $P=0.05$, ICA vs. MCA $P=0.83$). In the young group CPT evoked a greater velocity response in the MCA compared to the PCA (17±14% vs 10±10%, $P<0.01$), whereas no difference was seen in the older group (4±7% vs 3±7%, $P=0.72$).

Figure 4 provides the CVC values for the MCA, PCA, CCA and ICA during baseline, CPT and recovery in young and older participants. A significant interaction between age and experimental phase was observed for MCA CVC. Although MCA CVC was numerically lower in the older group across all experimental phases, post hoc analyses showed only a trend towards an age difference at CPT2 (P=0.07) with no significant differences from baseline in either group.

Table 2 presents arterial stiffness indices for the CCA and ICA. Arterial stiffness in the CCA was greater in the older group compared to the young individuals, whereas ICA stiffness was not different. No index of arterial stiffness was altered during the CPT.

DISCUSSION

The first major novel finding of the present study is that in young individuals there is a differential response to the CPT within the extra-cranial blood vessels (CCA vs. ICA) and also discrepant responses between the extra- and intra-cranial circulations. The second major novel finding is that in older individuals there is a blunting of the extra- and intra-cranial responses to the CPT. The physiological and clinical significance of these findings are considered below.

1) *Extra- and intra-cranial blood flow regulation during the cold pressor test:*

In accordance with earlier work in young individuals (53) we observed a significant increase in CCA diameter during the CPT. However, in contrast, and in accordance with our hypothesis, we observed no change in ICA diameter during the CPT. Despite this lack of change in ICA diameter and only a transient increase in ICA flow during the first minute of CPT, we observed that the CPT evoked a marked and persistent increase in $MCAv_{mean}$ – a finding in contrast to Bramanti *et al.* (3), but in agreement with several previous studies (46, 47, 56, 73). This may imply a differential regulation of the extra- and intra-cranial arteries that could serve an important protective role. There is evidence that the extra-cranial arteries (at the level of the ICA and vertebral arteries) are integral to the regulation of cerebral blood flow through modifying vascular resistance (14, 22, 29, 39, 40). Furthermore a MRI study reported decreased cerebral blood volume in response to sympathoexcitatory reflexes (69). In response to a sympathetically mediated hypertensive insult, the buffering function of the larger cerebral and large pial arterioles, but not the cerebral microcirculation, serves as a first line of defense in regulating cerebral perfusion pressure. Our data

315 indicate that the responses of the CCA are different from the ICA and MCA during
316 the CPT, at least in younger individuals.
317 Elevations in sympathetic vasoconstrictor activity and MAP produced by the
318 CPT have at least three effects on cerebral blood flow. First, is the obvious hydraulic
319 effect of MAP that increases flow even if vascular resistance is unchanged. Second,
320 and the one commonly either neglected or misunderstood, is the autoregulatory effect
321 of an increase in perfusion pressure to increase vascular resistance and minimise the
322 increase in flow. A likely third effect is the influence of SNA on extra- and intra-
323 cranial blood flow regulation. Thus, appreciation of the effects of the CPT on factors
324 such as the hydraulic effect and potential shear patterns of elevations in MAP, as well
325 as the concomitant changes in SNA and autoregulation, likely explain the apparent
326 differential mechanisms of regulation apparent between the CCA→ICA→MCA.
327 Although the sympathetic regulation of the cerebral blood vessels in humans
328 remains a controversial issue (1, 58), we did observe a decrease in MCA CVC (a
329 finding consistent with other studies (21, 52)) and demonstrate for the first time that
330 the CPT reduces CCA, ICA and PCA CVC. These latter changes in CVC are possibly
331 indicative of sympathetically-mediated cerebral vasoconstriction or autoregulatory
332 mediated. Bramanti *et al.* (3) demonstrated a reduction in $MCAv_{mean}$ during the CPT
333 (by ~23%) the magnitude of which was approximately halved following intrathecal
334 administration of the α_2-adrenergic receptor agonist clonidine. These findings support
335 the role of a central noradrenergic mechanism in the cerebrovascular responses to the
336 CPT. However, although not measured in this study, differences in $PaCO_2$ may
337 explain these conflicting findings. In the present study, a dynamic end-tidal forcing
338 system was used in an attempt to maintain $PetCO_2$ near baseline, thus permitting the

effect of the generalized sympathetic activation associated with the CPT to be observed.

Along with $MCAv_{mean}$ we determined the $PCAv_{mean}$ responses to CPT. There are known anatomical and physiological differences between anterior and posterior circulations. For example, the PCA may have less sympathetic innervation than the anterior cerebral portion (12, 20) and CO_2 reactivity is reduced (51). We observed that the temporal pattern of response $PCAv_{mean}$ and $MCAv_{mean}$ was similar, however interestingly the magnitude of response was greater in the MCA compared to the PCA in the young (17±14% vs. 10±10%).

2) *Blunting of the extra- and intra-cranial responses during the CPT in older individuals*:

In contrast to the younger group, the changes in both the extra and intra-cranial resistance and flow were generally blunted in the older group during the CPT. This is significant because dysfunctional CCA and coronary artery responses to the CPT have been associated with atherosclerotic disease (45, 53, 72). Since the MAP 'stimulus' or hydraulic effect was comparable, it seems reasonable that the differential extra and intra-cranial responses in young and older individuals reflect some fundamental differences in potential shear patterns induced via the elevations in MAP, as well as the influences of SNA, humoral factors, endothelial vasodilator function, autoregulation and parasympathetic control. Rubenfire *et al.* (53), speculated that a β-adrenergic mechanism accounted for the increase of CCA diameter during the CPT in healthy individuals, whereas the reduction in CCA diameter in coronary artery disease patients was due to greater sensitivity of the α-adrenergic receptors. This shift from a β-adrenergic vasodilatory response to an α-adrenergic vasoconstrictor one may be

related to underlying endothelial damage and dysfunction (71). Endothelial dysfunction is well established to occur within the peripheral vasculature of healthy elderly individuals and its extension to the cerebral vasculature might explain the present findings. Age-related alterations in arterial stiffness may also have contributed to the cerebrovascular responses reported. CCA stiffness was elevated in the older individuals at baseline, but in accordance with previous literature none of the calculated arterial stiffness indices was modified by the CPT (28, 33). Unfortunately, on the basis of our data set we cannot delineate the mechanism(s) for the blunting of the extra- and intra-cranial responses during the CPT in older individuals, but our findings provide direction for future studies.

3) Methodological considerations:

There are a number of methodological considerations that should be considered in the context of our study and related interpretation of the findings.

a) Discrepancies of flow and velocity during the CPT: The assessment of cerebrovascular responses during a myriad of physiological interventions has been dominated by the use of transcranial Doppler over the last 30 years. However, this approach operates on the assumption (also its primary limitation) that the insonated vessel (PCA, MCA) remains at a constant diameter. Older studies have partially corroborated that under various stimuli (e.g., orthostasis, CO_2 changes), $MCAv_{mean}$ accurately reflected the magnitude of changes in MCA blood flow as diameter remained unchanged (55), however, recent high resonance imaging studies have challenged this assumption of constant vessel diameter during marked changes in $PaCO_2$ or PaO_2 (8, 9, 64) or exercise-induced sympathetic activation (63). Furthermore, as recently reviewed (24), it is not known if the MCA diameter changes

389 during elevations in blood pressure. At least during hypertension (35) and
390 hypotension (32), discrepancies between ICA flow and $MCAv_{mean}$ have been reported.
391 Similarly, in the present study we observed that the percentage increase in ICA flow
392 was less marked than $MCAv_{mean}$ during the CPT. The effects of CO_2 and blood
393 pressure on PCA diameter are unknown.
394 *b) Flow vs. conductance:* To account for MAP in the analysis of extra vs.
395 intra-cranial cerebrovascular responses, CVC is commonly used. However, as
396 outlined above, increases in MAP produced by the CPT may affect cerebral blood
397 flow by several independent and interacting mechanisms (e.g., hydraulic effect,
398 autoregulation, shear stress). As such, CVC is not likely to accurately account for the
399 CPT-induced elevations in MAP during the CPT, and consideration of these
400 mechanisms will be needed to fully understand the apparent differential regulation of
401 the CCA→ICA→MCA.
402 *c) CPT recovery:* We included recovery data in our analyses to verify that the
403 cardiovascular, respiratory and cerebrovascular variables of interest returned to
404 baseline following the CPT. In all instances the measured parameters did successfully
405 recover. Interestingly, an elevated systolic blood pressure recovery from the CPT is an
406 important predictor of a future elevation in systolic blood pressure (57). Whether there
407 is any prognostic significance to the cerebrovascular response to or following the CPT
408 remains to be investigated.
409 *d) Study limitations*: Roatta *et al.* (52), reported that the $MCAv_{mean}$ increases
410 during hand CPT were slightly but significantly greater on the contralateral side
411 (+4.4%) compared to the ipsilateral side (+2.4%). However, as the aim of our study
412 was to simultaneous assess CCA, ICA, MCA and PCA responses to the CPT
413 measurements were necessitated on both the contralateral and ipsilateral sides, thus

414 unfortunately it was not practical to account for any potential lateralization of the
415 cerebral hemodynamic response to the CPT. In addition, hydration status was not
416 assessed, which may be a limitation as this has recently been reported to modify the
417 cerebrovascular response to the CPT (47). We cannot exclude the possibility that age-
418 related differences in thermoreceptor sensitivity contributed to the CPT responses we
419 observed (15), although ratings of perceived pain were not different in the young and
420 old groups during the CPT. One older individual was taking the α_{1A} adrenoreceptor
421 antagonist tamsulosin for an enlarged prostate. Although these receptors are present in
422 the ureter, they are less well expressed in the peripheral vasculature (44, 50). This
423 individual displayed cerebral perfusion similar responses to the rest of the older
424 group, and their removal did not affect the results of the statistical analyses.
425 It should be noted that our findings can only be directed to young and older
426 healthy volunteers and that the regulation of cerebral blood flow may further differ in
427 patients with cerebrovascular disease. Nevertheless, to be able to interpret the
428 pathophysiological significance of these observations, a clear understanding of the
429 normal responses of the cerebral circulation must first be obtained before extension
430 can be made to pathological groups. Given that risk factors for coronary artery disease
431 are associated with the extra-cranial blood vessel responses (53), future studies should
432 explore the cerebrovascular responses in individuals at risk or in those that have
433 experienced cerebrovascular events.
434
435 *4) Clinical implications:*
436 The CPT has been widely employed for cardiovascular risk stratification (6,
437 38, 61). Likewise, an attenuated cerebrovascular reactivity is indicative of an
438 increased risk for all cause and cardiovascular (inclusive of stroke) mortality (48). The

magnitude of the vasomotor response in the extracranial (ICA, vertebral artery) and intracranial arteries (MCA, PCA) to a CPT perturbation may be indicative of cerebrovascular health (i.e., endothelial function), much like peripheral flow mediated dilation is indicative of cardiovascular risk (18, 25). Thus future studies are needed to further explore vasomotor responses to CPT in individuals at risk of or who have experienced cerebrovascular events. Consequently, the CPT may serve as a simple diagnostic tool to predict cerebrovascular events and reduce related disabilities and mortality.

In conclusion, during the CPT, for the first time we reveal; 1) differential mechanism(s) of regulation between the ICA compared to the CCA in young individuals; 2) a blunting of the extra- and intra-cranial responses in older individuals; and 3) irrespective of age, there were discrepancies in the magnitude of change in CCA flow, ICA flow and $MCAv_{mean}$ during the CPT.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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FIGURE CAPTIONS

Figure 1

Middle cerebral artery mean blood flow velocity ($MCAv_{mean}$) and posterior cerebral artery mean blood flow velocity ($PCAv_{mean}$) in young (n=10, black circles) and old (n=9, n=8 for $PCAv_{mean,}$ grey triangles) at baseline (BL), during a three-minute cold pressor test (CPT1, CPT2, CPT3) and followed by a three-minute recovery (RE1, RE2, RE3). Values are means±SEM. *P* values represent repeated two-way ANOVA results. **P* < 0.05 vs. BL; ^{d} *P* < 0.05 vs. CPT1; $^{+}$ *P* < 0.05 vs. CPT2; § *P* < 0.05 vs. CPT3.

Figure 2

Common carotid (CCA) diameter, internal carotid (ICA) diameter, CCA velocity, ICA velocity, CCA flow and ICA flow in young (n=10, black circles) and old (n=9, n=7 for ICA, grey triangles) at baseline (BL), during a three-minute cold pressor test (CPT1, CPT2, CPT3) and followed by a three-minute recovery (RE1, RE2, RE3). Values are means±SEM. *P* values represent repeated two-way ANOVA results. **P* < 0.05 vs. BL; ^{d} *P* < 0.05 vs. CPT1; $^{+}$ *P* < 0.05 vs. CPT2; § *P* < 0.05 vs. CPT3.

Figure 3

Percentage change from baseline (BL) in common carotid (CCA) flow, internal carotid (ICA) flow, and middle cerebral artery mean blood flow velocity ($MCAv_{mean}$) in young (n=10, black symbols) and old (n=9, n=7 for ICA, grey symbols) during a three-minute cold pressor test (CPT1, CPT2, CPT3) and followed by a three-minute recovery (RE1, RE2, RE3). Values are means±SEM. *P* values represent repeated two-way ANOVA results.

Figure 4

Middle cerebral artery cerebrovascular conductance (MCA CVC), posterior cerebral artery (PCA) CVC, common carotid (CCA) CVC and internal carotid (ICA) CVC, in young (n=10, black symbols) and old (n=9, n=7 for ICA, grey symbols) at baseline (BL), during a three-minute cold pressor test (CPT1, CPT2, CPT3) and followed by a three-minute recovery (RE1, RE2, RE3). Values are means±SEM. *P* values represent repeated ANOVA results. **P* < 0.05 vs. BL; ^{d} *P* < 0.05 vs. CPT1; $^{+}$ *P* < 0.05 vs. CPT2; § *P* < 0.05 vs. CPT3

TABLES

Table 1. Cardiovascular and respiratory parameters at baseline (BL), at each minute of a three minute cold pressor test (CPT1, CPT2, CPT3) and at each minute during a three-minute recovery (RE1, RE2, RE3).

		Experimental phase							*P* values		
		BL	CPT1	CPT2	CPT3	RE1	RE2	RE3	Age	Phase	Age* Phas
MAP (mmHg)	Y (n=10)	93±7	109±9	108±10	104±9	96±7	93±7	94±9	**0.026**	**<0.001**	0.79
	O (n=9)	101±7	114±10	114±9	112±8	104±6	102±5	103±5			
HR (bpm)	Y (n=10)	73±12	81±18	77±17	73±15	67±12	69±13	68±11	0.105	**<0.001**	0.26
	O (n=9)	64±9	68±9	67±6	65±6	62±8	61±9	61±9			
SV (ml)	Y (n=10)	96±42	93±42	90±41	90±42	94±43	94±42	95±42	0.271	**0.046**	0.85
	O (n=9)	93±30	89±28	88±28	86±26	89±27	90±27	87±27			
CO ($l \cdot min^{-1}$)	Y (n=10)	7.4±1.1	8.0±2.0	7.3±1.6	7.2±1.3	6.8±1.1	7.0±0.9	6.9±1.0	**0.005**	**0.002**	0.50
	O (n=9)	5.6±2.0	5.5±1.5	5.4±1.4	5.1±1.3	5.1±1.5	5.2±1.5	5.0±1.6			
TPR (mmHg· min ml^{-1})	Y (n=10)	12.8±1.9	14.4±3.7	15.3±3.1	14.9±2.8	14.3±2.6	13.5±2.2	13.8±2.3	**0.003**	**<0.001**	0.80
	O (n=9)	20.4±7.3	22.9±7.6	22.9±6.8	23.6±6.3	22.3±6.8	21.5±6.5	22.6±6.8			
VE ($l \cdot min^{-1}$)	Y (n=10)	14.6±3.8	18.4±5.4	18.2±5.0	18.1±4.8	16.1±3.7	14.8±3.1	15.1±3.2	**0.038**	**<0.001**	0.27
	O (n=9)	12.1±3.6	14.5±4.5	13.7±3.4	13.2±3.9	11.7±3.6	11.4±3.6	11.7±3.3			
$PetCO_2$ (mmHg)	Y (n=10)	41.5±2.8	41.2±2.4	41.3±2.9	41.4±2.9	41.3±2.7	41.4±2.7	41.0±2.4	0.201	0.212	**0.04**
	O (n=9)	40.1±2.5	39.65±3.0	39.3±3.0	39.1±2.8	39.9±2.7	39.7±2.5	**40.2±2.7**[§]			

Abbreviations: MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; VE, ventilation; $PetCO_2$, end-tidal partial pressure of CO; Y, young; O, old. Values are mean±SD. *P* values represent two-way repeated ANOVA results (Age: young and old; Phase: BL, CPT1, CPT2, CPT3, RE1, RE RE3). [§]$P < 0.05$ versus CPT3.

Table 2. Arterial stiffness indices in young and old individuals at baseline (BL) and during the cold pressure test (CPT)

		Young	Old	*P* - value		
				Age	Phase	Age*Phase
CCA β stiffness	BL	5.5±1.1	7.6±2.3	**0.001**	0.980	0.843
	CPT	5.6±1.1	7.5±1.9			
ICA β stiffness	BL	8.5±4.2	6.7±2.6	0.676	0.635	0.419
	CPT	8.0±4.9	8.6±2.2			
CCA Elastic modulus (mmHg)	BL	555.9±129.5	796.3±198.2	**<0.001**	0.131	0.910
	CPT	636.9±126.1	890.2±219.1			
ICA Elastic modulus (mmHg)	BL	846.6±384.4	698.4±264.9	0.879	0.227	0.383
	CPT	896.5±496.2	1000.8±269.5			
CCA Arterial compliance ($cm \cdot mmHg^{-1}$)	BL	0.012±0.003	0.010±0.003	**0.023**	0.191	0.998
	CPT	0.011±0.002	0.009±0.002			
ICA Arterial compliance ($cm \cdot mmHg^{-1}$)	BL	0.008±0.003	0.011±0.006	0.499	0.856	0.256
	CPT	0.009±0.005	0.009±0.005			
CCA Arterial distensibility ($mmHg^{-1}$)	BL	0.002±0.000	0.001±0.001	**0.002**	0.234	0.947
	CPT	0.002±0.000	0.001±0.000			
ICA Arterial distensibility ($mmHg^{-1}$)	BL	0.002±0.001	0.002±0.001	0.467	0.562	0.343
	CPT	0.003±0.006	0.001±0.000			

Abbreviations: CCA, common carotid artery; ICA, internal carotid artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; BL, baseline; CPT, cold pressor test. *P* values represent two-way repeated ANOVA results (Age: young and old; Phase: BL, CPT1, CPT2, CPT3, RE1, RE2, RE3)